

REMARKS

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

Date

4/4/03

By



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Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 19-0741 for any such fees; and applicants hereby petition for any needed extension of time.

MARKED UP VERSION ATTACHED TO AMENDMENT IN

SERIAL NO. 09/887,669

Marked up version of the paragraph starting at page 11, lines 24-34 is below:

FIG. 3 shows the predicted amino acid sequence (SEQ ID NO: 1) of the RPTP_K precursor protein. The putative signal peptide and transmembrane (TM) segment are underlined. The two tandem phosphatase domains are boxed (PTP-1, PTP-2). The proteolytic cleavage site (RTKR, residues 640-643 of SEQ ID NO: 1) is printed in bold, and the Ig-like domain (Ig, 214-270) shown in bold italic characters. A5: homology to A5 surface protein (Takagi, S. et al., 1991 Neuron 7:295-307); FN-III: fibronectin type III repeats. The Genbank accession number for the cDNA sequence is L10106.

Marked up version of the paragraph starting at page 11, lines 24-34 is below:

Please delete the paragraph on page 11, line 35 to page 12, line 10, and replace it with the following paragraph:

FIG. 4 shows a proposed alignment of the four FN-III repeats of RPTP_K (residues 296-383 of SEQ ID NO: 1, residues 392-473 of SEQ ID NO: 1, residues 493-578 of SEQ ID NO: 1, and residues 596-679 of SEQ ID NO: 1, respectively, in order of appearance) and domain 7 of human fibronectin (SEQ ID NO: 5) (Kornblihtt, A.R. et al., 1985 EMBO J. 4:1755-1759). Residues most typically conserved in FN-III repeats are highlighted in bold. Residues identical in three or more out of the five aligned sequences are indicated with an asterisk. This region of the protein also contains clearly detectable homology to LAR, *Drosophila* PTPase 10D, and *Drosophila* neuroglian, all of which have been reported to contain FN-III repeats.

Marked up version of the paragraph starting at page 12, lines 11-19 is below:

FIG. 5 shows an alignment of the N-terminal domains of RPTP κ (residues 33-189 of SEQ ID NO: 1) and mRPTP μ (SEQ ID NO: 6) with the cell surface protein A5 (SEQ ID NO: 7) (Takagi *et al.*, *supra*). Numbers indicate the first residue of the respective proteins shown in the alignment. Residues marked as consensus are identical between A5 and RPTP κ , or between A5 and mRPTP μ . Conservative substitutions are present but not shown. Residues in bold (C,W) define a possible Ig-like domain structure.

Marked up version of the paragraph starting at page 14, lines 6-13 is below:

FIG. 11 shows the effect of mutagenesis of the furin cleavage motif RTKR (SEQ ID NO: 12) on RPTP κ processing. Total lysates from mock-transfected COS cells, cells expressing wt RPTP κ , or RTKR (CM κ) (SEQ ID NO: 12) were resolved by SDS-PAGE. Immunoblotting was performed using anti-N-terminal antiserum 116 (left panel), or anti-cytoplasmic antiserum 122 (right panel).

Marked up version of the paragraph starting at page 15, lines 1-9 is below:

FIG. 14 is a molecular model describing the processing of the R-PTP- κ precursor protein. A furin-like endoprotease cleaves the 210 kDa precursor protein, after which both cleavage products (110 and 100 kDa) remain associated. No suggestions as to the mechanism of association are intended. The numerals 116 and 122 designate the sites of epitopes recognized by antisera described in the text. The RTKR peptide is shown in SEQ ID NO: 12.

Marked up version of the paragraph starting at page 66, lines 8-15 is below:

In vitro site-directed mutagenesis was performed using a commercially available kit from Clontech, using the manufacturer's instructions. An oligonucleotide having the sequence CTACACCCACATCTAACGAACCGTGAAGCAGGG (SEQ ID NO: 11) was used to modify the amino acid sequence RTKR (SEQ ID NO: 12) in the cleavage site to the sequence LTNR (SEQ ID NO: 13). Mutagenesis was confirmed by direct DNA sequencing.

Marked up version of the paragraph starting at page 73, lines 5-11 is below:

The present inventors noted the presence of a proteolytic cleavage signal in the extracellular domain of RPTP_K, (RTKR, residues 640 to 643 of SEQ ID NO: 1), in the fourth FN-III repeat; FIG. 3) and wished to examine its significance in light of these observations. Thus, additional experiments were performed in COS cells transfected by the DEAE-dextran technique.

Marked up version of the paragraph starting at page 74, lines 14-26 is below:

The above results, as well as pulse-chase analysis shown in FIG. 10, are consistent with the cleavage of a 210 kDa RPTP_K precursor protein into an N-terminal 110 kDa product encompassing most of the extracellular domain, and a 100 kDa moiety containing the intracellular portion and about 100 residues of extracellular sequence (FIG. 14). A consensus site for cleavage by furin, a processing endopeptidase (Hosaka *et al.*, *supra*), is indeed located 113 amino acids upstream of the start of the transmembrane segment (RTKR, residues 640-643 of SEQ ID NO: 1), which would leave one potential N-glycosylation site in the C-terminal cleavage product.

Marked up version of the paragraph starting at page 74, lines 27-36 is below:

In order to confirm directly that proteolytic cleavage occurred at the RTKR (SEQ ID NO: 12) (furin-recognized) site, site-directed mutagenesis was used to mutate this site to LTNR (SEQ ID NO: 13), and the effects of this mutation on the processing of the RPTP_K precursor were examined. As shown in FIG. 12, the mutant cDNA gave rise to only a 210 kDa product. These results provide evidence that the RTKR (SEQ ID NO: 12) region is the likely proteolytic cleavage signal and site for processing, of the RPTP_K proprotein.

Marked up version of the paragraph starting at page 79, line 18 to page 80, line 9 is below:

MCP7 is highly homologous to mRPTP_u which has a more restricted expression pattern in lung, brain and heart (Gebblink et al., *supra*). MCP7 is expressed as a molecule consisting of two noncovalently linked subunits, a structural feature already shown for LAR. A similar processing motif was also determined within the extracellular domain of mRPTP_u (RTKR, [residues 632-635] SEQ ID NO: 12), which suggest that this structural organization is typical for the family of type II phosphatases. Proteolytic cleavage also occurs in the extracellular domain of the cell adhesion molecule Ng-CAM in a region containing the dibasic processing motif (Burgoon, M. P. et al., *J. Cell. Biol.* 112:1017-1029 (1991)). The functional significance of this structure is not yet clear. For LAR, a shedding of the extracellular E-subunit was observed in a density-dependent manner (Streuli et al., *supra*). It is likely that this shedding is due to a conformational change in the extracellular domain caused by homophilic or hydrophilic interactions between the molecules on the surface of neighboring cells that weakens the interaction between the noncovalently linked subunits. The effect of this shedding on the activity of the PTPase domains within the cells is not yet clear, but a modification of the activity of the phosphatase or a change in the sensitivity to modifying processes is probable.